apoptosis/survival (M6.6) and inflammation (M4.6) modules. We show an inverse relationship between HIV-specific responses (production of IL-2, IL-13, IL-21, IFN-γ, CD4 polyfunctionality, i.e. production of at least two cytokines) and the peak of viral load during ATI. Those cellular immune responses were positively correlated to genes associated with T cell functional modules (M4.1, M4.15) at w16 and negatively correlated to genes associated with inflammation (e.g. EGLIN1, H2AFY, LTBR). Specifically, IL-21 secretion, that was associated with control of viral replication, was negatively correlated to MFAP3 and TFNRSF1A inflammatory genes.
Conclusion: Changes in gene expression profile were associated with vaccine-elicited cellular responses and viral rebound during ATI. Integrated analysis led to identify DC vaccine signatures likely correlated with a better control of HIV replication.

OA02.01
Engineering an HIV Envelope Protein to Activate Germline B Cell Receptors of Broadly Neutralizing VRC01-Class Antibodies

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Background: The recent RV144 trial showed ~30% efficacy. Although the protection was modest, the trial indicated for the first time that a vaccine against HIV is possible. Immune correlate analysis suggests that the observed protection was due to non-neutralizing antibody responses. This efficacy might be improved if a vaccine could elicit broadly neutralizing antibodies (bNAbs). Of particular interest for vaccine design are the potent VRC01-class bNAbs targeting CD4 binding site on Env. Unfortunately, a number of studies have demonstrated that recombinant Env proteins do not bind germline-reverted VRC01 class Abs, indicating that current vaccine strategies using recombinant Env are unable to activate progenitor B cells that ultimately give rise to VRC01 class Abs.
Methods: We developed reagents and experimental protocols to express functional versions of the mature and germline VRC01 and NIH45–46 BCRs on the surface of B cells, and assays to monitor B cell activation following the antigenic-engagement of these BCRs.
Results: Although several recombinant Env proteins were capable of binding to and stimulating B cells expressing the mature BCR forms, no such interactions were recorded with B cells expressing the germline BCR forms. However, we identified key conserved glycosylation sites in the Loop D and V5 regions of a Clade C Env that prevent the binding of the clonally related germline NIH45–46 and germline VRC01 BCRs. Disruption of these glycosylation sites resulted in nM binding affinity to germline BCRs and activation of the corresponding B cells.
Conclusion: Our study identifies the earliest roadblock in the elicitation of anti-CD4-BS bNAbs; the lack of engagement of the germline BCR forms of 'VRC01' class antibodies by commonly used HIV Env immunogens. Importantly, we have developed a way to overcome this roadblock through the design of an HIV Env that engages and activates B cells expressing the germline BCR forms of 'VRC01' class antibodies.

OA02.02
Focusing Antibody Responses to Specific Protein Surfaces Through Site-Selective Glycan Addition

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Background: Viruses such as human immunodeficiency virus type-1 (HIV-1) and influenza A virus display rapidly mutating or non-neutralizing immunodominant epitopes on their viral glycoproteins to escape antibody-mediated neutralization.
Methods: Immunosilent glycoconjugates with terminal sialic acid were added site-selectively to the model protein hen egg lysozyme (HEL), mutant an HIV-1 gp120 or engineered gp120 outer domain. Epitope mapping was subsequently performed on sera from immunized mice.
Results: Glycoconjugate addition of HEL led to site-selective loss of antibody binding to epitopes containing modification sites in vitro. Immunization with modified protein led to refocusing of antibody responses from masked to unmasked epitopes in vivo. Application of this sialic acid masking strategy to HIV antigens led to loss of antibody recognition in masked regions whilst maintaining antigenicity of the CD4 binding site which is a target of broadly neutralizing antibodies.
Conclusion: Our masking strategy attaches relatively small glycoconjugates to properly folded proteins under mild conditions with near 100% efficiency. This approach circumvents many of the problems, such as improper folding and low occupancy, associated with masking strategies using mutational redistribution of N-linked glycosylation sites. Thus, sialic acid glycoconjugate masking should allow focused targeting of specific antigenic regions for increased B cell recognition, improving vaccine antigen design.

OA02.03
Comparative Antigenicity and Immunogenicity of Indian and South African HIV-1 Subtype C Native and CD4 Liganded Envelope Glycoproteins

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Background: The ability to induce a potent and broadly neutralizing antibody (bNAb) response following vaccination is critical in developing an effective HIV-1 vaccine. This study describes the design and construction of three HIV-1 subtype C South African (derived from founder virus sequences) and three Indian (derived from circulating virus sequences) Env immunogens, and their antigenicity and immunogenicity testing in the presence or absence of liganded CD4, in rabbits.
Methods: Monomeric (gp120) and trimeric (gp140GCN4+) conformations for IN26191, IN25710, IN25925, ZACAP45, ZACAP210 and ZA706010164 were expressed in mammalian cells. Unliganded or 2dCD4 S60C liganded Env glycoproteins were purified by lectin affinity chromatography, followed by conformation and complex purification using size exclusion